



# The Virtual Institute of Microbial Stress and Survival

## Rapid Deduction of Stress Response Pathways in Metal/Radionuclide Reducing Bacteria

## Applied Environmental Microbiology Core



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## Overview



### AEMC Mission

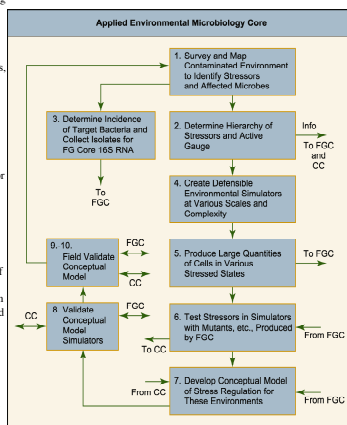
AEMC is the source of environmental data and samples that determine the stressors that will be studied, provides the environments for growing the organisms to be tested, simulates stressed environments, and verifies the conceptual models to determine how these stress regulatory pathways control the biogeochemistry of contaminated sites

### Main Goals

- Develop criteria for monitoring the integrity (health) and altering the trajectory of an environmental biological system (process control).
- More complete understanding of the diversity and environmental context of stress response.

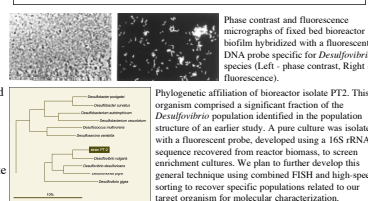
### Specific Aims

- Survey and map DOE sites contaminated by metals and radionuclides using chemical and molecular microbiological parameters to determine major microbial populations and potential stressors for *Desulfovibrio vulgaris*, *Geobacter metallireducens*, and *Shewanella oneidensis* MRI.
- Determine the rank priority of these stressors in terms of their ability to affect metal/radionuclide bioreduction by either direct or indirect processes, and to establish the normal active range of the stressor in metal/radionuclide contaminated environments.
- Determine the incidence and activity of the three target bacteria, and closely related relatives, in the test metal/radionuclide contaminated environments and collect isolates for analysis by the Functional Genomics Core for comparison using 16S RNA profiling.
- Create defensible environmental simulators that can replicate key features of field site chemical and biological structure to mimic stress conditions for single populations and later for microbial communities (chemostats to soil columns from 10 µm to 1 m size systems).
- Provide large quantities of cells in various stress states for the Functional Genomics Core's physiological monitoring facility, and molecular interaction studies.
- Provide environmental simulators for testing stressor effects on mutants, large insert clones, expression analysis, etc., for elucidating critical parts of the stress regulation pathway.
- Develop testable conceptual models of stress regulatory pathways based on results of the Computational Core that could predict natural attenuation and suggest biostimulatory strategies for immobilization of metals and radionuclides at DOE contaminated sites.
- Test conceptual models of stress regulatory pathways and effects on contaminant site biogeochemistry using competent soil columns with different levels of complexity over the active range of the stressors
- Validate conceptual models using field tests at contaminated sites that utilize specific functional gene arrays developed from the stress regulatory pathways.
- Alter field conditions or test along gradients to verify stress regulatory model efficiency for predicting natural attenuation or suggesting biostimulatory strategies for immobilization of metals and radionuclides.



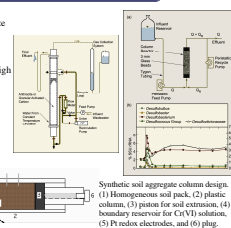
## Environmental Simulator Stress Analyses

- Growth and recovery of stressed single populations under simple conditions
- Identification and recovery of stress-responsive populations from complex communities
- Radiomicroarray analysis
- Recovery of stress responsive populations via flow cytometry
- Flow cytometric sorting of FISH-labeled populations
- Expression analysis of RNA extraction from sediment columns and reactor systems
- Synchrotron FTIR direct analysis of stress changes in living cells
- Direct stress and community comparison with PLFA and metabolite analyses



## Reactor System Development

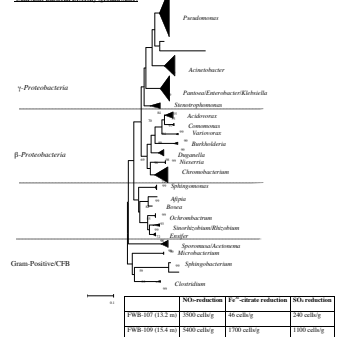
- Analogous to saturated soil/sediment systems in which the microbiota colonize surfaces as biofilms
- Growth as biofilms is the more common state for microorganisms in the environment
- Fluidized bed reactor systems provide for experimental control, replication, high biomass, and ease of sampling
- Biofilm-associated populations are more resistant to stress and are the more relevant state for most environmental populations
- Biofilm-associated populations are not washed out (of the reactor system) following imposition of stress, providing for greater range of stress-response experimentation
- Past reactor studies have shown that *Desulfovibrio* spp. and *Geobacter*-like populations will colonize and be retained within these systems
- Chemostat Studies
- Cultural isolation of organisms for comparative "Benchmarks"
- Collection of environmental samples for reactor inoculation
- Comparability of reactor and field site community structure



## Chemical & Molecular Microbiological Mapping of Field Sites

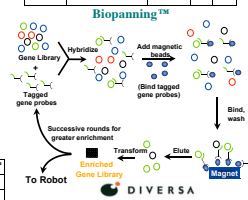
- Survey of existing data at the NABIR Field Research Center at Oak Ridge - Draft report completed.
- Additional sediment and soil sampling for: physical/chemical stressors, wild isolates of target bacteria, and TRFLP and clonal population mapping (Biopanning™ in progress).

Substrate Specificity: Desulfovibrio



Sample	Substrate Specificity	Groundwater
DP22	Desulfovibrio	Desulfovibrio
DP11	Desulfovibrio	Desulfovibrio
TMW07	Desulfovibrio	Desulfovibrio
TMW09	Desulfovibrio	Desulfovibrio
TMW12	Desulfovibrio	Desulfovibrio
FW300	Desulfovibrio	Desulfovibrio
GW355	Desulfovibrio	Desulfovibrio

Site	16S rRNA	Species	OT	OT
FW-100	100	Desulfovibrio	100	100
FW-101	101	Desulfovibrio	101	101
FW-102	102	Desulfovibrio	102	102
FW-103	103	Desulfovibrio	103	103
FW-104	104	Desulfovibrio	104	104



## Validation of Conceptual Models with Simulators and Field Tests

This research element will not begin until the 3rd year after pathways have been identified and tested.

## Experimental Core Facilities

**Environmental Molecular Microbiology Facility** (ORNL, LBNL, U. Wash., Diversa Inc.): Affymetrix microarray system, PLFA analysis lab, Radio-microarray lab, low capacity DNA microarray, flow cytometer, Laser confocal microscope lab, Synchrotron FTIR beamline (Advanced Light Source), clone libraries from NABIR FRC, Microarrays for *Shewanella*

**Environmental Simulation and Culture Facility** (LBNL, U. Wash., Diversa Inc., U. Missouri): Anaerobic chambers with built-in microscopes and incubators, instrumented soil columns (various sizes), Ultracold freezers & liquid nitrogen archiving, Biopanning™, Chemostats (Fermentis), Batch Fermentors, Fixed bed reactors, Fluidized bed reactors, radiological control areas for microbiological sample prep and incubation